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In re patent application of
Pierre CHAMBON *et al.*

Serial No. 09/853,033

Group Art Unit: 1636

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Examiner: Celine X. QIAN

Title: TRANSGENIC MOUSE FOR TARGETED RECOMBINATION MEDIATED BY
MODIFIED CRE-ER

DECLARATION UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Pierre H. Chambon, hereby declare that:

1. I am a co-inventor on the above-captioned application. I have held the position of Director of the Institut Clinique de la Souris since 2002. My qualifications are set out in my *curriculum vitae*, which is attached hereto as APPENDIX A.
2. Many genes and proteins encoded by these genes are expressed and function in different cell types and at different stages of development and adult life, and may be implicated in different pathological states. Therefore, an important tool for understanding the function of given genes in different processes would be the ability to delete or modify a gene at will in a given tissue during a particular stage of development or adult life in whole animal models.
3. The mouse is currently considered the most appropriate mammalian model because of similarities between the mouse and human genomes, the amount of genetic and biochemical data available, the relative ease of maintaining experimental colonies, and the experimental tools that have been developed.
4. The present invention provides a method for efficient stage- and tissue-specific modification of a given endogenous gene in its natural genomic environment in the living mouse. At the time of filing, examples of such modifications in three cell populations (keratinocytes, adipocytes and hepatocytes), from three distinct organs, using different transgenic mice

expressing fusion proteins were provided. Each of these fusion proteins contained a modified estrogen receptor ligand binding domain (ER^T , ER^{T2}) conferring *in vivo* tamoxifen-inducibility to the activity of the fused Cre recombinase. These transgenic lines were then bred with mice bearing endogenous genes, in their normal chromosomal position and environment, that contained engineered LoxP sites specifically recognized by Cre recombinase. Thus, upon tamoxifen treatment, the fused Cre recombinase specifically deleted, in the progeny, gene segments flanked by LoxP-sites.

5. In each case, the deletion was 100% efficient following tamoxifen treatment in all cells in which the recombinase was expressed. Moreover, a deletion was not observed in the absence of tamoxifen, demonstrating that the inventive method permitted tight temporal control of the generation of cell type / tissue-specific somatic mutations.

6. Since that time, I and my collaborators, as well as others have constructed additional mouse lines that efficiently express tamoxifen-activated $Cre-ER^T$ and $Cre-ER^{T2}$ fusion proteins in a variety of cell types/tissues, and allow efficient spatio-temporally-controlled targeted somatic mutagenesis in the mouse, confirming the generality of the present invention. The instant invention provides the investigative tool necessary for the dissection of gene function in a whole animal model.

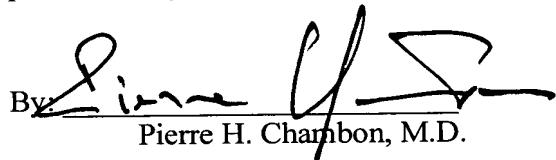
7. Clearly, achieving tightly temporally-controlled somatic mutations in the mouse, targeted to endogenous genes in their normal chromosomal position and environment, was not obvious at the time of filing from published work on Cre-ER fusion proteins.

8. Indeed, Feil *et al.* (Proc. Natl. Acad. Sci. USA 98, 10887-10890, 1996) and Indra *et al.* (Nucl. Acid. Res. 27, 4324-4327, 1999) only demonstrated that $Cre-ER^T$ or $Cre-ER^{T2}$ fusion proteins could be used to delete DNA segments within synthetic reporter transgenes.

9. Additionally, Schwenk *et al.* (Nucl. Acid. Res. 26, 1427-1432, 1998), demonstrated that a tamoxifen-inducible Cre-ER fusion protein could be used to delete a chromosomal DNA segment, but with variable and incomplete recombination efficiency. Indeed, high doses of tamoxifen had to be used to reach at best 80% deletion such that the tamoxifen anti-oestrogenic

activity could become harmful for the mouse. Accordingly, the tamoxifen-activated Cre-ER fusion protein of Schwenck *et al.* could not be used to delete a chromosomal DNA segment with an efficiency sufficient to validly study gene function.

10. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By 
Pierre H. Chambon, M.D.

Date: Sept 25th 2003